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Immune modulation of biologic systems in renal somatic cells

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Components of the classical immune response mediate most traditional acts of destructive inflammation in body tissues. These events normally begin with the presentation of new peptide antigens to helper T lymphocytes by antigen-presenting cells residing within the lymphoid system. This engagement results in the elaboration of new lymphokines, and/or the emergence of new effector T cells and antibodies. These same developmental processes and events are also thought to occur when antigens are first recognized in the renal parenchyma. Not all immunologic responses within the kidney, however, are necessarily inflammatory.

The biology of somatic renal cells, for example, may only be phenotypically altered after contact with the immune system, and such contact may take place in the absence of any obvious injury. The immune response, therefore, may sometimes largely act as a signaling messenger for biologic change. Antibodies or antigen-binding soluble factors mediating these biologic changes would be specifically targeted, theoretically, to ligands expressed on certain cell types. These immune mediators are highly focused in their interaction and, depending on the second messenger signals, would produce phenotypic alterations in the target cell which are either protective or detrimental to the host. Some of the mechanisms whereby the immune system can engage and biologically alter renal somatic cells are the focus of the following review.

Antigen-binding T cell proteins

The characterization of soluble antigen-binding proteins or T-helper factors (ThF) provides a predictable mechanism for the induction of effector T cell responses in the absence of cell-to-cell contact. Such factors are mature, soluble end-products which can functionally substitute for their respective parent lymphocytes [1, 2]. They are produced by T cells, are usually antigen-specific, and traditionally engage the effector limb of an immune response [3–8]. Both T and B cell differentiation may be enhanced by such factors [9]. In contrast, antigen-binding suppressor proteins may do the opposite [9–12]. Previous biochemical characterizations of various helper factors have established that these molecules are commonly composed of two chains. One chain functions as an antigen-binding domain while the other expresses class II MHC determinants, thereby providing immunologic restriction [2, 3, 7, 9, 13–16].

At least one ThF has been described in experimental interstitial nephritis [2]. This factor induces the nephritogenic cell-mediated effector response in anti-tubular basement membrane (αTBM) disease, demonstrates fine antigenic specificity, and is composed of two molecular chains [2, 16]. The ThF is derived from specific $CD4^+$ T-helper cells reactive to 3M-1 antigen, the 30,000 M_r glycoprotein autoantigen of αTBM disease [17].

In this experimental model, genetically susceptible SJL mice ($H2^s$, IgH-1b) develop tubulointerstitial nephritis after immunization with renal tubular antigen in complete Freund's adjuvant [18–29]. The 3M-1 target antigen is synthesized by proximal tubular cells (MCT) and in vivo is later distributed along cortical tubular basement membranes [17, 19, 20, 21]. $CD4^+$ T helper cells that are both 3M-1 antigen-specific and I-A restricted induce the appearance of $CD8^+$ nephritogenic effector cells and polyclonal anti-3M-1 antibodies [18, 22, 23, 26–30].

ThF is secreted by the $CD4^+$ helper cell and can be isolated as the non-covalently bound chains of a heterodimeric molecule. One chain binds 3M-1 and this antigen-binding chain expresses components of the T cell receptor (TcR) as evidenced by its ligation by several anti-T cell receptor-like antibodies [2]. Monoclonal antibody 14-30 also binds the framework region of the ThF at a determinant common to other antigen-binding factors, while another monoclonal antibody αM30 recognizes a portion of the α:β heterodimeric TcR [27]. Other antibodies which bind to this chain include KJ23, an antibody specific for Vβ17, and an anti-idiotypic antibody raised against antibodies eluted from nephritis mice [31]. KJ23 recognizes a domain shared by the 3M-1 idiotypic network which includes a soluble T cell suppressor factor, and effector cells [32–33]. The second chain of the ThF dimer (M_r ~82,000) expresses Aβ determinants, the murine equivalents of human class II MHC molecules, and binds to an anti-$A\beta^s$ monoclonal antibody.

ThF performs two known functions, the first is immunological and the second is biological. In the first, ThF promotes a dose-dependent induction of $CD8^+$ effector cells, in the presence of IL-2 and 3M-1 [2]. Notably, the immunologic helper function of ThF is not perturbed even after physical disruption of its component chains through chemical alterations of alkylation and reduction [2]. The second and more novel function of this antigen-binding protein is its ability to selectively alter molecular and cellular events within proximal tubular cells.

MCT cells in culture, as an example, secrete several collagens into their supernatants, including collagen types I, III and IV [34, 35]. Culture of these cells with various concentrations of ThF leads to differential effects on the secretion of types I and IV collagen into the supernatants [36]. After 72 hours of culture, a 20-fold dose-dependent decrement in type IV collagen secretion can be observed. In contrast, the secretion of type I collagen is either unchanged or slightly enhanced by ThF [36].

The decrement in type IV collagen secretion strikingly contrasts the increases seen after the incubation of MCT cells with other cytokines and cytokine-like paracrine modulators, including TGFβ, EGF and other antigen-specific T cell helper factors [2, 26].

The mechanism by which ThF down-regulates type IV collagen secretion is partially understood. In vitro experiments measuring the relative abundance of transcripts encoding collagen chains αI(IV) and α2(I) in MCT cells after stimulation with ThF revealed a threefold reduction in mRNA encoding the αI(IV) chain [36]. By contrast, a slight elevation in the number of transcripts occurs with type I collagen. In vitro nuclear run-off studies demonstrate that the principal reason for the reduction in levels of mRNA encoding type IV collagen can be attributed to an overall decrease in its rate of transcription. In renal tissues of mice with early αTBM disease, T cells that stain for 3M-1 antigen-reactive ThF are found within the interstitial spaces. In situ hybridization studies of such nephritic kidneys also demonstrate a parallel reduction in transcripts encoding type IV collagen [36]. This decrease in transcripts is reflected in lower amounts of type IV collagen in basement membrane as interstitial injury progresses.

The primary constituents of tubular basement membrane are type IV collagen and laminin. The renal tubular epithelium sits on this laminar support, suspended by a fibrillar collagen scaffolding that partitions the compartments of the tubulointerstitium [37–39]. One consequence of releasing antigen-binding proteins like ThF into the interstitium is a substantial decrease in type IV collagen. Alterations in the cellular expression of extracellular matrix collagens by ThF-like moieties can directly facilitate the deformative remodeling of the tubulointerstitium during interstitial inflammation, and may provide an immunologic basis for the process of tubular atrophy and fibrogenesis [34, 36, 40, 41].

Antibody against renal cells

Antibodies to Heymann nephritis antigen complex

A paradigm for the modulating influence of antibody against antigen on glomerular epithelial cells (GEC) is Heymann nephritis, an experimental model of membranous nephropathy [42]. In the active form of disease, immunization of rats with whole kidney homogenates in adjuvant produces a heavy proteinuria. In the passive form, proteinuria is induced by the injection of either autologous or heterologous antibodies or $F(Ab)'_2$ fragments that recognize a cellular antigen called Fx1A/HNAC/gp330, currently referred to as the Heymann nephritis antigen complex (HNAC) [personal communication, Dr. M.G. Farquhar; 43–47]. In both the active and passive forms of the disease, subepithelial immune deposits form along the subepithelial aspects of the GBM [46–50]. The interaction of antibody with HNAC in the tuft is not directly injurious to the GBM, since proteinuria occurs only after local complement activation; however, administration of passively transferred antibodies against brush border antigens can invoke changes along the proximal tubule in the absence of complement activation [51].

More recently, it has been observed that the antigen complex recognized by anti-HNAC antibodies (αHNAC-Ab) are likely subunits of a large, 400 to 600,000 M_r multimeric membrane-associated glycoprotein (HNAC) that is found with restricted polarity on the brush border of proximal tubular cells in the clathrin-rich inter-microvillar domain of the cell membrane and in the glomerular epithelium at the soles of the foot processes where the cell membrane faces the GBM [52–58].

Immunohistochemical localization of HNAC to the glomerular epithelial cell (GEC) has caused a reappraisal of the pathogenic mechanism of this disorder. Originally, it had been presumed that circulating immune complex formation occurred after circulating αHNAC-Ab became bound to small amounts of tubular brush border antigen that had spontaneously leaked from the kidney and entered the serum [43]. These complexes would deposit within the GBM in a subepithelial distribution and initiated disease. Currently, however, it is believed that the formation of immune deposits occurs largely in situ [37, 50, 59]. αHNAC-Ab somehow traverse the GBM and then bind avidly to HNAC in the clathrin-coated pits at the base of podocytes and subsequently form immune deposits [60–63]. The association of HNAC with clathrin, its partial similarity to brushin [58], and its homology to the LDL receptor suggests that the true nature of the complex, multimeric molecule is still uncertain [58, 64, 65]. The association of antibody with HNAC is highly specific, however, and certain epitopes are capable of inciting greater nephritogenic responses than others. For example, polyclonal brush border antibody preparations with high reactivity for HNAC produce greater proteinuria than isolated monospecific fractions [60, 66, 67]. Within these heterogeneous preparations, gp330-specific antibodies have been shown to be the most nephritogenic [58]. Cell-surface binding of αHNAC-Ab serves as the nidus for the subsequent development of subepithelial immune complexes which are shed into the lamina rarae externae and subsequently stabilized by crosslinking [47]. Aggregation of immune complexes leads to formation of large immune deposits which take form within 15 minutes and may persist in the GBM for up to eight days [47]. In culture, clustering and shedding of immune deposits occurs preferentially in the presence of polyclonal immune serum, rather than monoclonal serum, indicating that binding of multiple epitopes may occur. This arrangement likely optimizes lattice formation for the ensuing complement activation which occurs in Heymann nephritis [60, 67–69]. The enlargement of immune deposits is dependent on the continued biosynthesis of gp330 which is transported from the endoplasmic reticulum and Golgi apparatus to sites of immune interaction near the clathrin-coated pits [58]. Persistence of the immune deposits in the lamina rara externa of the GBM may occur because of ongoing exposure of nephritogenic epitopes to αHNAC-Ab within the immune complexes. Furthermore, the association of gp330 with clathrin is so strong that it is maintained even after isolation of brush border membranes by biochemical fractionation [58, 61].

With persistence of immune deposition in Heymann nephritis, significant thickening of the GBM proceeds [70]. Progressive thickening follows remodeling of the GBM structure itself [71–74]. However, the remodeling process is unusual in that there is no obvious increase in the synthesis in type IV collagen, laminin, or fibronectin [70]. Measurement of mRNA levels for these matrix components does not increase when measured from isolated glomeruli at either one week, three weeks, or three months [70]. Whether there is an increase in some other

undefined structural matrix constituent is not known. Candidates for such a component would include entactin and proteoglycans. Alternatively, a volumetric increase in the GBM may have taken place without concomitant new matrix synthesis, namely, the density of the membrane becomes decreased [70].

The nephritogenicity of HNAC may depend, in part, on expression of conformational epitopes not normally visible in vivo. After ligation by polyclonal antibodies, but not monoclonal antibodies, HNAC undergoes antigenic modulation [47, 48]. HNAC is subsequently redistributed on the apical membrane surface, patched, actively capped, and temporarily internalized after initial cross linking [47, 48]. These processes are influenced by cytoskeletal components and may be linked to one another, affecting the differential expression of epitopic specificity.

The cytoskeleton of a cell supports and provides its infrastructure. It operates dynamically and responsively to the microenvironment, in conjunction with a cell's molecular motors. As an example, the cytoskeleton behaves relatively passively when monovalent Fab' fragments of αHNAC-AB bind to Heymann nephritis antigens on the cell surface [45]. However, after binding of αHNAC-Ab and crosslinking of HNAC, the cytoskeleton becomes engaged in new actions. HNAC is redistributed and bound by αHNAC-Ab that form aggregates or "caps." These subsequently redistribute within the cell membrane [60, 75]. This capping phenomenon, which is an active process dependent on the presence of divalent cations, can be precluded by pretreatment of GEC with cytochalasin B which disrupts microfilaments, or by the addition of a calcium ionophore [75, 76]. Like capping, antigenic redistribution is also sensitive to the administration of colchicine which disrupts microtubular structures [77]. After cross linking of HNAC by αHNAC-Ab, these antigen-antibody complexes subsequently disappear from the cell membrane of the podocyte. Their subsequent reappearance in the subepithelial space at sites of slit diaphragms implies some sort of translocation to this region [47, 48].

Using differential extraction techniques, it has been shown that a conversion of HNAC from the stage of a detergent-soluble, membrane-associated form to an insoluble cytoskeleton-bound one occurs in the presence of αHNAC-Ab [76]. In the latter conformation, HNAC is tightly associated with microfilaments, thereby gaining mobility within the cytoplasm. Although the exact role of the association of the cytoskeleton with HNAC is not defined by these observations, it is likely that its role is fundamental to further antigen processing, since the administration of chlorpromazine in vitro and in vivo completely abrogates the development of Heymann nephritis [75]. Chlorpromazine inhibits the actions of calmodulin and protein kinase C and mediates a decrease in the fluid nature of the cell membrane through its effects on microfilaments [75].

The induction of proteinuria by monoclonal αHNAC-Ab is rapid, reversible and transient. This interaction is specific and does not proceed when a monoclonal antibody directed against a smaller 70,000 M_r subunit of the 600,000 M_r multimer is substituted for polyclonal αHNAC-Ab [55]. This latter antibody may interact synergistically with HNAC by increasing the permeability of the endothelium, thereby permitting a greater quantity of αHNAC-Ab to traverse the GBM, gaining access to the surface of GEC [60]. Furthermore, polyclonal αHNAC-Ab is not uniquely nephritogenic in Heymann nephritis; a distinct antibody directed against the 108,000 M_r subunit of the 600,000 M_r multimer seems to elicit an even more severe nephritogenic response than polyclonal αHNAC-Ab [77]. Unfortunately, the molecular mechanism which governs the alteration of glomerular permeability in Heymann nephritis remains uncertain.

Interestingly, recent data also suggest a role for αHNAC-Ab in the alteration of cell adhesion of proximal tubular epithelium to the extracellular matrix. Proximal tubular epithelium in primary culture attach to their substratum and propagate as a differentiated structure [78]. These effects are mediated, in part, by HNAC. The detergent-solubilized HNAC fraction of freshly isolated epithelium has specific affinity for extracellular matrix components, including type I collagen, laminin and fibronectin, which is reminiscent of cell receptor β1 integrins [79–81]. However, the affinity for these matrix components is neither blocked by the tripeptide sequence, RGD (arginine-glycine-asparagine), nor by synthetic fibronectin peptides, implying a non-integrin binding mechanism for HNAC. This is not unexpected since the distribution of HNAC on glomerular epithelium differs from that of the β1 integrins. The inhibition of attachment of this epithelium to these matrix constituents in vitro by αHNAC-Ab strongly contends that a receptor epitope of HNAC mediates part of this adhesion [78]. Furthermore, the inhibition is not secondary to a toxic effect since the epithelial attachment to these matrix components is fully restored after removal of antibody from the cell surface. Another immunobiological effect of αHNAC-Ab is the prevention of the expected increase in thymidine incorporation by tubular epithelium after their culture on extracellular matrices [78].

A disturbance of proximal tubular endocytosis in rats is also mediated by αHNAC-Ab. After the injection of heterologous HNAC, there is a pronounced diminution in the endocytic uptake of FITC-conjugated dextran and horseradish peroxidase [82]. Loss of microvillar structures and flattening of the epithelial cells occurs [58]. Immunocytochemical analysis discloses a coincident and parallel reduction in the amount of several apical surface antigens, including HNAC, clathrin and an ATP-dependent proton pump. Clathrin and antigen-antibody complexes can be found in the tubular lumen. Since clathrin-coated pits play a major role in the endocytosis of filtered proteins in proximal tubules, it is not improbable that the latter phenomenon contributes to the tubular dysfunction which attends Heymann nephritis.

Antibodies to other glomerular epithelial antigens

Several other experimental models may also provide insight into potential signaling mechanisms using immunoglobulin recognition of glomerular epithelial cells. In one model in rats, a single intravenous injection of the monoclonal antibody 5-1-6 (α5-1-6) predictably produces an immediate massive proteinuria in a dose-dependent fashion [83]. The inciting antigen has been localized exclusively to rat kidney and defines the organ specificity of this interaction. Protein excretion peaks after eight days and then gradually declines. Immunoperoxidase studies demonstrate a linear binding pattern along glomerular capillary walls within two hours after injection of α5-1-6. By the third day, this pattern is succeeded by a fine granular pattern of fluorescence whose intensity diminishes by twelve days. This

change in immunofluorescence implies mobility of the autoantigen and quite plausibly, molecular rearrangement of immune complexes. Correlative ultrastructural studies have noted antibody *within* multivesicular bodies and localization to the surface of foot processes at slit diaphragms, similar to HNAC. The intracellular presence of antibody contrasts the mechanism of induction of Heymann nephritis where much of the antibody is seemingly not internalized. Histologically, there is partial retraction of epithelial foot processes. Taken collectively, these data imply that the determinants recognized by α5-1-6 directly participate in the maintenance of the permselective function of the glomerular capillary walls. Moreover, the structural alterations therein might initiate or augment the mechanism(s) causing proteinuria. The lesions produced by α5-1-6 recapitulate the histopathological findings attendant in other experimental models of non-inflammatory glomerulonephritis to include nephrosis induced by adriamycin, systemic protein overload and by the aminonucleoside puromycin [84–91].

In the K9/9 nephritis model, even more impressive structural alterations in GEC appear after the administration of this monoclonal IgG antibody. This immunoglobulin recognizes a specific sialoglycoprotein in Lewis rat kidney which leads to rapid and reversible proteinuric response. K9/9 immunoprecipitates *both* a 115 kD sialoglycoprotein of the glomerular cell membrane and a 107,000 M_r membrane component from proximal tubules [92]. Leukocytes and complement are not involved in the mechanism of proteinuria; however, there is an absolute requirement of the nonselective activation of the immune system by adjuvant for expression of the response. The structural alterations which appear include microvillous transformation, effacement of foot processes, vacuolar transformation and epithelial cell detachment from the GBM [92]. This model defines a specific nephritogenic epitope since antibodies which bind to spatially close but different epitopes cannot elicit a similar nephritogenic response [93].

In both of these models, it appears that immune recognition of epithelial ligands interferes with the orderly contact and interface of structural elements which comprise normal tissue planes. Antibody engagement subsequently disrupts the functional activity of these elements in their support of the microenvironment.

Antibodies to proximal tubular cells

Antibodies to determinants expressed by proximal tubular cells can also have an effect on MHC class II expression [94]. In αTBM disease, for example, susceptible strains of SJL mice (H-2^s, IgH-1b) develop both class I-restricted $CD8^+$ and class II-restricted $CD4^+$ effector cells early in disease [17, 22, 23, 26, 28–30]. Within several days, however, class I-restricted effector cells emerge as the predominant phenotype, and adoptive transfer of these particular effectors will produce disease [24].

By contrast, the non-susceptible strain of B10.A (H-2^a, IgH-1b) mice develops a class II-restricted $CD4^+$ effector cell response [24]. Although this effector cell phenotype recognizes 3M-1 in delayed-type hypersensitivity assays, it is normally not nephritogenic. Such class II-restricted cells, however, can mediate tubulointerstitial inflammation upon adoptive transfer into syngeneic recipients whose tubular epithelium have been primed by an appropriate cytokine. For example, pretreatment of B10.A mice with rγIFN augments the expression of class II antigens on proximal tubular epithelium, rendering this strain susceptible to interstitial nephritis [94]. Prior to such treatment, class II antigens are present in insufficient quantities on 3M-1-secreting tubular cells to mediate the T cell recognition of 3M-1. This is not surprising, since the normal expression of class II by proximal tubular cells is minimal [94–96]. However, under appropriate conditions, tubular epithelium can present antigen [25, 94–97], and we believe that rγIFN permissively increases the immunologic visibility of 3M-1, thus enhancing its intrinsic antigen-presenting capacity.

It appears that the permissive effect of rγIFN can be abrogated by the passive transfer of antibodies to 3M-1 [94]. B10.A mice pretreated with α3M-1-Ab and rγIFN 48 hours before receipt of effector $CD4^+$ cells prevented induction of a nephritogenic response [94]. Protection was afforded by α3M-1-Ab even when administered as late as 24 hours before adoptive transfer. Immunofluorescent staining of class II molecules on the tubules from B10.A mice was minimal in comparison to mice which were exposed to an irrelevant control antibody, where the latter stained abundantly for class II. Additional studies suggested that the protective effect of α3M-1-Ab was not simply due to the interference of T cell recognition of antigen, since α3M-1-Ab could not provide a protective response in syngeneic recipients of adoptively transferred 3M-1-reactive, $CD8^+$ effector cells.

Kidneys from recipients of passively transferred α3M-1-Ab also stain TBM with increased intensity when overlain with additional α3M-1-Ab at the time of immunofluorescent analysis. Taken together, these data suggest that passively transferred α3M-1-Ab neither completely blocked T cell epitope recognition, nor influenced the expression of class I determinants [94].

Further studies have examined the mechanism by which α3M-1-Ab down-regulates class II. These investigations reveal a diminution in levels of mRNA transcripts for class II determinants in MCT cells incubated with α3M-1-Ab for 24 hours. Similar effects were obtained when polyclonal IgG eluates from kidneys of susceptible B10.S and non-susceptible B10.A strains were incubated with MCT cells. Kidney-bound α3M-1-Ab from either strain down-regulated the transcript levels for class II antigens. Unexpectedly, however, exposure of MCT cells to the haplotype specific anti-class I antibody αK^s nearly tripled class II mRNA levels [94].

Nuclear run-off transcription assays performed on MCT cells cultured in the presence of α3M-1-Ab pinpoint the modulatory effect of α3M-1-Ab to an inhibition of class II gene activation [94]. A 50% decrease in the rate of class II mRNA transcription was observed with α3M-1-Ab in comparison to levels obtained from cells incubated with control antibody. There was no effect on class I transcription. In situ hybridization studies of kidneys of mice immunized to produce αTBM disease corroborated the in vitro findings. At two or five weeks, the period during which α3M-1-Ab are first deposited along the tubular basement membrane and before there is a strong mononuclear reaction, one-sixth to one-third of the mRNA levels of class II MHC are expressed by mice immunized to produce disease in comparison to controls [94]. Furthermore, the tubules from mice with αTBM disease demonstrate a much heavier deposition of endogenous α3M-1-Ab than those from controls.

In summary, α3M-1-Ab binding to proximal tubular cell surface 3M-1 selectively down-regulates the expression of class

II MHC determinants by repressing transcription. The functional and biological effects result in a diminution of cell surface class II expression that attenuate $CD4^+$-mediated T cell responses. Presumably the lack of a response is the result of a failure in antigen presentation by the proximal tubular epithelium.

Summary

The various theories discussed here suggest that somatic renal cells are susceptible to biologic modulation by the immune system independent of an inflammatory effect. (1) The mode of repression of type IV collagen synthesis by novel, soluble antigen-binding proteins, the down-regulation of class II MHC expression with interruption of antigen presentation to epithelia after selective gene regulation by antibody, and the diverse interactions of antibody with renal glomerular cells producing functional disturbances in endocytosis and permselectivity; (2) modification of surface-antigen composition; (3) alteration of matrix deposition, remodeling and composition; (4) biophysical perturbation of cytoskeletal and cell membrane components; (5) and lastly, alterations in cell adhesion through cell-surface alterations, all lend testimony to the richness of the signal transduction pathways in somatic cells. Although the preceding examples represent only a small fraction of those which may take place within the glomerular and tubular microenvironments, these paradigms may nevertheless serve as new models upon which one can consider the multitude of potential communications between disparate biologic systems that connect in complex organisms.

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